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Butanol production from wood pulping hydrolysate in an integrated fermentation–gas stripping process

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HIGHLIGHTS

- Wood pulping hydrolysate (WPH) was used for butanol production by *C. beijerinckii*.
- Resin adsorption was effective to remove inhibitors in the hydrolysate.
- Batch fermentation with gas stripping for butanol separation alleviated butanol toxicity.
- ABE production reached 17.7 g/L, the highest ever achieved in fermentation with WPH.
- This process can be used to produce butanol from the abundant wood pulping waste.

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ABSTRACT

Wood pulping hydrolysate (WPH) containing mainly xylose and glucose as a potential substrate for acetone–butanol–ethanol (ABE) fermentation was studied. Due to the inhibitors present in the hydrolysate, several dilution levels and detoxification treatments, including overliming, activated charcoal adsorption, and resin adsorption, were evaluated for their effectiveness in relieving the inhibition on fermentation. Detoxification using resin and evaporation was found to be the most effective method in reducing the toxicity of WPH. ABE production in batch fermentation by *Clostridium beijerinckii* increased 68%, from 6.73 g/L in the non-treated and non-diluted WPH to 11.35 g/L in the resin treated WPH. With gas stripping for *in situ* product removal, ABE production from WPH increased to 17.73 g/L, demonstrating that gas stripping was effective in alleviating butanol toxicity by selectively separating butanol from the fermentation broth, which greatly improved solvents production and sugar conversion in the fermentation.

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1. Introduction

Acetone–butanol–ethanol (ABE) fermentation, one of the oldest industrial fermentation, has received renewed interests for the past several decades in search of a sustainable bio-based n-butanol (Jin et al., 2011). In recent years, concerns of depleting oil reserves and rising oil prices have prompted the demand for renewable fuels to increase the fuel independency of a country. Biobutanol has demonstrated its superiority to bioethanol in terms of energy density, engine compatibility and safety, and has become the center of research as the next generation biofuel since 2005 (Nigam and Singh, 2011).

Raw material has been the most cost-intensive part of ABE fermentation; it greatly influences the butanol production cost and thus the process economics and feasibility (Green, 2011; Gu et al., 2011). Biobutanol from food-based substrates such as corn and molasses is considered the first-generation biofuel, whereas biobutanol from inedible biomass as the second-generation biofuel has attracted the most research efforts in the recent years (Nigam and Singh, 2011; Weber et al., 2010). Lignocellulosic biomass represents the largest renewable carbon source on the earth, including agricultural residues, energy crops, forestry woody residues and municipal solid wastes (Kumar et al., 2009). Successful utilization of lignocellulosic biomass hydrolysates usually requires detoxification as many fermentation inhibitors are produced under the extreme conditions during pretreatments (Jang et al., 2012; Jurgens et al., 2012). Energy crops, such as switchgrass, and agricultural residues, such as barley straw, wheat straw, corn fiber, and corn stover, have been successfully used as substrates for ABE fermentation (Qureshi et al., 2007, 2008, 2010a,b). Green liquor, which contains mostly dissolved hemicellulose and lignin from wood chips in the paper pulping industry, is an abundant resource of carbohydrates available, but has not been well studied for butanol production.

Aside from raw material, the biggest limiting factor in ABE fermentation has been butanol toxicity and inhibition (Ezeji et al., 2010; Gu et al., 2011). Many *in situ* butanol recovery techniques,

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including pervaporation, liquid–liquid extraction, adsorption and gas stripping, have been reported to relieve butanol inhibition and enhance the efficiency of ABE fermentation (Vane, 2008; Yang and Lu, 2013). Among them, gas stripping is an effective technique with low energy requirement and easy to integrate with the fermentation process, and has been demonstrated for *in situ* butanol recovery from the ABE fermentation broth to alleviate butanol toxicity and inhibition on the fermentation (Ezeji et al., 2004; Lu et al., 2012; Qureshi et al., 2007; Xue et al., 2012).

In this work, the green liquor containing hemicellulose from a paper pulping plant was investigated as a potential low-cost substrate for butanol production. Our aims were to evaluate the feasibility of using wood pulping hydrolysate (WPH) for ABE fermentation by *Clostridium beijerinckii* and to develop a process for producing renewable and green n-butanol from WPH. The effectiveness of several detoxification techniques for removing fermentation inhibitors and their effects on ABE fermentation were investigated. In addition, gas stripping was employed as an *in situ* recovery method to alleviate butanol toxicity and enhance butanol production and sugar conversion in the fermentation.

2. Methods

2.1. Wood pulping hydrolysate

WPH, obtained from a wood pulp mill, contained mainly xylose (42.74 g/L), glucose (20.84 g/L), and some arabinose (1.97 g/L), with a total sugar content of 65.54 g/L. The hardwoods, mainly from maple, beech and birch trees, used in the pulp mill contained approximately 40–55% cellulose, 24–40% hemicellulose and 18–25% lignin on the dry weight basis. The WPH was prepared as follows. Wood chips were first cooked, washed and extracted of hemicellulose and lignin in alkaline for paper pulping. The dissolved hemicellulose along with lignin in the green liquor was hydrolyzed with sulfuric acid at an elevated temperature to release sugars, mainly xylose and glucose. The hydrolysate containing about 2–3% (w/v) of total sugars was then concentrated by evaporation to ~6.5% (w/v) total sugars with its pH adjusted to around 2 to 3 for easier transfer and storage. Some WPH was treated with ion exchange resins to remove inhibitors present in the hydrolysate before evaporation. For non-detoxified WPH, overliming and activated charcoal adsorption were evaluated for their effectiveness in removing inhibitors and relieving their inhibition on fermentation. Overliming was carried out by adding $Ca(OH)_2$ to adjust the pH of WPH to 10.0, followed by heating to 90 °C and agitation at 100 rpm for 30 min. After removing the precipitates by centrifugation at $7000\times g$ for 10 min, the solution was cooled to the room temperature and the pH was adjusted back to 6.5 by adding concentrated H_2SO_4. Finally, the supernatant was collected after removing new precipitates formed in pH adjustment by centrifugation at $7000\times g$ for 10 min. Detoxification with activated charcoal adsorption was carried out by adding 2 g of activated charcoal to 100 ml of WPH at pH 2.0, and heating to 90 °C with agitation at 150 rpm for 30 min. Afterwards, activated charcoal was removed from WPH by vacuum filtration. The compositions of various WPHs are given in Table 1.

2.2. Culture and media

C. beijerinckii CC101, an adaptive mutant strain of *C. beijerinckii* NCIMB 8052 (ATCC 51743) obtained by adaption in a fibrous bed bioreactor and culture selection in our lab was used as the working culture for ABE fermentation. Spores of *C. beijerinckii* CC101 were stored in the refrigerator at 4 °C in the clostridia medium. Spores (2 ml) were heat-shocked at 80 °C for 3 min and transferred to 50 ml reinforced clostridia medium (RCM, Difco, Becton, Dickinson and Company, MD, USA) in a 125-ml serum bottle containing the P2 medium described below. After purging with nitrogen for 8 min to remove oxygen, the bottle was capped with a rubber stopper and aluminum seal, and then autoclaved at 121 °C for 30 min. After cooling to 37 °C, the bottle was inoculated with heat-shocked spores and incubated at 37 °C for 12–16 h to obtain highly active cells, which were used as seed culture for fermentation kinetics study. Unless otherwise noted, all fermentation studies were carried out with the P2 medium containing a carbon source (glucose, xylose or WPH), yeast extract (2 g/L), buffer (0.5 g/L KH_2PO_4 and 0.5 g/L K_2HPO_4), 2.2 g/L ammonium acetate, vitamins (1 mg/L *p*-amino-benzoic acid, 1 mg/L thiamin and 0.01 mg/L biotin), and mineral salts (0.2 g/L $MgSO_4\cdot 7H_2O$, 0.01 g/L $MnSO_4\cdot H_2O$, 0.01 g/L $FeSO_4\cdot 7H_2O$, 0.01 g/L NaCl). Unless otherwise noted, the carbon source (sugars or WPH), minerals, vitamins, and the remaining components in the P2 medium were prepared in separate bottles and mixed aseptically after sterilization (Lu et al., 2012).

2.3. ABE fermentation in serum bottles

ABE fermentation with WPH as the main substrate was studied first in serum bottles each containing 50 ml medium to evaluate the effects of various detoxification methods on butanol production. To ensure the medium pH maintained above 5.0 throughout the fermentation, 2 g/L of $CaCO_3$ was also included in the medium. After adjusting the medium pH to 6.5 using 6N NaOH, actively grown *C. beijerinckii* CC101 cells were inoculated at 5% (v/v) and the culture was incubated at 37 °C with no agitation. Samples were taken periodically for analysis of sugar consumption and ABE production.

Table 1
Compositions of the untreated WPH and detoxified WPHs used in this study.

Concentration (g/L)	WPH, untreated	WPH, detoxified by overliming	WPH, detoxified by activated charcoal adsorption	WPH, detoxified by resin adsorption
Glucose	20.84 ± 1.84	17.80 ± 1.76	20.15 ± 0.72	11.80 ± 0.65
Xylose	42.74 ± 5.10	35.71 ± 1.63	41.09 ± 2.8	47.18 ± 2.39
Arabinose	1.97 ± 0.48	1.83 ± 0.29	1.21 ± 0.24	2.60 ± 0.86
Total sugars	65.54 ± 6.62	55.34 ± 3.07	62.45 ± 2.67	61.58 ± 3.46
Acetic acid	2.95 ± 1.38	2.46 ± 0.2	2.17 ± 0.24	0
Formic acid	0.27 ± 0.06	0.21 ± 0.01	0.19 ± 0.02	0.20 ± 0.04
Furfural	0.34 ± 0.05	0.30 ± 0.02	0.068 ± 0.004	0.29 ± 0.03
HMF	0.076 ± 0.009	0.014 ± 0.004	0.009 ± 0.001	<0.001
Ferulic acid	<0.001	<0.001	<0.001	<0.001
Levulinic acid	1.22 ± 0.13	0.96 ± 0.06	<0.001	0.92 ± 0.05
p-Coumaric acid	<0.001	<0.001	<0.001	<0.001
Phenolics	0.019 ± 0.007	0.036 ± 0.007	<0.001	0.008 ± 0.002

2.4. ABE fermentation in bioreactor

Batch fermentation with WPH was studied in a 5-L stirred-tank bioreactor with the pH controlled at 5.0 by adding 6N NaOH. No $CaCO_3$ was used and the medium in the bioreactor was purged with nitrogen through a 0.2-μm filter for 2 h after sterilization. Batch fermentation was also studied with gas stripping for *in situ* butanol recovery, which was done by continuously circulating the fermentation gases (H_2 and CO_2) at 1.25 L/min through the fermentation broth in the bioreactor and a glass coil condenser (Pyrex Brand Graham Condenser, 300 mm water jacket, Fisher Scientific, NJ, USA) at ~1 °C using a peristaltic pump (Masterflex L/S, standard drive, Cole-Parmer, IL, USA). The gas recirculation was in a closed loop to minimize any vapor loss into air. The condensate was collected in a 125-ml conical flask cooling trap. The condenser and tubings used in the gas stripping system were autoclaved and purged with nitrogen before being aseptically connected to the bioreactor.

2.5. Analytical methods

Acetone, butanol, ethanol, acetic acid, and butyric acid in the fermentation broth samples were analyzed with a gas chromatograph (GC, Shimadzu GC-2014) equipped with a flame ionization detector and a 30 m fused silica column (0.25 μm film thickness and 0.25 mm ID, Stabilwax-DA). The carrier gas was nitrogen at 1.47 ml/min (linear velocity: 35 cm/s). Samples were diluted 20 times with an internal standard buffer solution containing 0.5 g/L isobutanol, 0.1 g/L isobutyric acid and 1% phosphoric acid (for acidification), and injected (1 μL each) by using an auto injector (AOC-20i Shimadzu). The column temperature was held at 80 °C for 3 min, raised to 150 °C at a rate of 30 °C/min, and held at 150 °C for 3.7 min. Both the injector and detector were set at 250 °C.

WPH and fermentation broth samples were analyzed for their sugar and organic acids contents using a high performance liquid chromatograph (HPLC) equipped with an organic acid analysis column (Bio-Rad HPX-87H) and a refractive index detector (Shimadzu RID-10A) at 45 °C, with 0.005 M H_2SO_4 as the eluent at 0.6 ml/min. HPLC analysis of potential organic inhibitors, including furfural, 5-hydroxymethylfurfural (HMF), levulinic acid, ferulic acid, *p*-coumaric acid and phenolic compounds, in WPH were carried out with Rezex ROA-Organic Acid H^+ (8%) column (Phenomenex) at 40 °C, with 0.005 M H_2SO_4 as the eluent at 0.6 ml/min. The detection was done with a UV/UIS detector (SPD-20AV, Shimadzu) at 280 nm for furfural, hydroxymethylfurfural (HMF) and phenolic compounds (with syringaldehyde as the standard), and at 200 nm for levulinic acid, ferulic acid, and *p*-coumaric acid.

3. Results and discussion

3.1. ABE fermentation in serum bottles

ABE production by *C. beijerinckii* CC101 using WPH as substrate was studied first in serum bottles to evaluate the effects of inhibitors or the degradation products, such as furfural, HMF, acetic acid and phenolic compounds produced from sugar, hemicelluloses and lignin during the pulp pretreatment and acid hydrolysis processes (Kumar et al., 2009). As these degradation products could be highly inhibitory to ABE fermentation, dilution and detoxification by overliming or adsorption with activated charcoal or resin were studied to evaluate their effects on improving the ABE fermentation. P2 medium with glucose and xylose as substrates was used as a control. The fermentation results are summarized and compared in Fig. 1 and Table 2. For the control, 13.67 g/L ABE was produced, of which 10.59 g/L was butanol. The corresponding ABE yield was 0.42 g/g and butanol yield was 0.32 g/g, which was higher than the typical butanol yield of 0.20–0.25 g/g in ABE fermentation. The higher butanol yield could be attributed to the lower acetone production by *C. beijerinckii* CC101, which gave a high butanol/acetone ratio of about 3:1 instead of the typical 2:1. Thus, more carbon flux was directed to butanol production and less for acetone production, which is a desired trait of *C. beijerinckii* CC101.

3.1.1. Effects of diluting WPH on ABE production

When using untreated and undiluted WPH as the carbon source, only 6.73 g/L ABE, a 50.8% reduction compared to the control, was produced with a large amount of sugars (31.14 g/L) remaining unused by the cells. The ABE yield also decreased to 0.29 g/g. It is clear that the ABE fermentation suffered from severe inhibition caused by the inhibitors present in the WPH (see Table 1). Therefore, diluting WPH, which reduced the inhibitor concentration, significantly improved butanol production and increased sugar utilization to 68.7%, compared to only 42.7% with the undiluted WPH (Fig. 1B). More ABE and butanol at higher yields were also obtained with the diluted WPH, increasing from 0.29 g/g to 0.38 g/g for ABE and from 0.19 g/g to 0.31 g/g for butanol (Table 2), which were almost identical to those from the control. Although dilution was effective to reduce the total concentration of the inhibitors and thus restore the ABE production to some extent, it was not sufficient, even at 50% dilution, to allow the cells to completely utilize all sugars in the medium to produce butanol to a comparable level to that for the control. Further dilution is undesirable as it would not provide a sufficient amount of sugars for the ABE fermentation to reach a high enough butanol concentration for economical recovery. In addition, since not all the sugars present in the concentrated WPH can be utilized in the ABE fermentation and dilution is needed, a less concentrated WPH with ~4.0% total sugars could be prepared and used in the fermentation, which would significantly reduce the energy consumption during the concentration step.

3.1.2. Effects of detoxification of WPH on ABE production

During the pretreatment and hydrolysis of lignocellulosic biomass, many compounds such as furfural, HMF, acetic acid, levulinic acid, formic acid, ferulic acid, *p*-coumaric acid and phenolic compounds could be generated at an inhibitory level. The WPH used in this study had little ferulic acid and *p*-coumaric acid (<0.001 g/L), but contained significant amounts of furfural, HMF, acetic acid, formic acid, phenolics and levulinic acid (see Table 1). Methods to remove these inhibitors from the WPH were thus investigated for their effects on butanol production. Compared to the untreated WPH, resin adsorption allowed more sugars to be converted to ABE and resulted in a 104% increase in butanol production (see Table 2). Activated charcoal adsorption also improved butanol production from WPH by ~40%, whereas overliming did not show any improvement. As can be seen in Table 1, overliming did not significantly remove the inhibitors, except for HMF, from WPH. Activated charcoal detoxification was effective in removing almost all of levulinic acid (99.9%) and phenolic compounds (99.9%), most of furfural (80.0%) and HMF (87.9%), but only a small fraction of formic acid (29.6%) and acetic acid (26.4%). In contrast, resin adsorption removed acetic acid completely, 99.9% HMF, 57.9% phenolics, 25.9% formic acid, 24.6% levulinic acid, and 14.7% furfural. It is clear that the removal of some of these inhibitors present in the WPH significantly improved ABE fermentation. The results also suggested that *C. beijerinckii* CC101 might be more sensitive to acetic acid and HMF than furfural, levulinic acid, and phenolics.

It should be noted that only a small amount of sugars was lost in the detoxification treatment with activated charcoal or resin adsorption, whereas overliming resulted in a 15.6% total sugar loss (see Table 1). Similar sugar loss (8.7 ± 4.5%) was also reported on

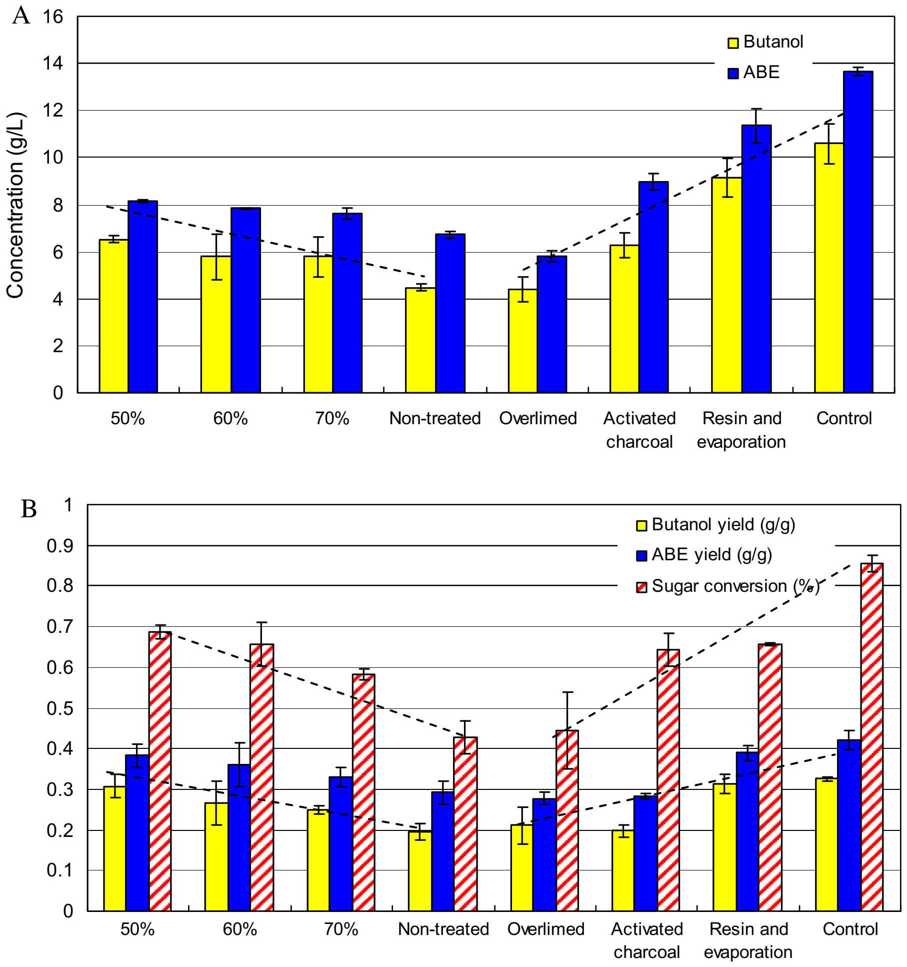

Fig. 1. Effects of dilution and different detoxification methods on ABE fermentation of WPH by *C. beijerinckii* CC101 in serum bottles. (A) Butanol and total ABE production; (B) Butanol yield, ABE yield, and sugar conversion.

Table 2
ABE production from WPH in batch fermentation by *Clostridium beijerinckii* CC101.

Fermentation conditions	Substrate	Initial sugar conc. (g/L)	Acetone (g/L)	Butanol (g/L)	ABE (g/L)	Butanol yield (g/g)	ABE yield (g/g)	Sugar conversion (%)	Butanol productivity (g/L h)	ABE productivity (g/L h)
Serum bottle	50% WPH	31.1 ± 1.4	1.30 ± 0.11	6.53 ± 0.15	8.14 ± 0.06	0.31 ± 0.03	0.38 ± 0.03	68.7 ± 1.8	0.09 ± 0.002	0.12 ± 0.001
	60% WPH	33.2 ± 1.8	1.81 ± 0.61	5.79 ± 0.97	7.83 ± 0.05	0.27 ± 0.05	0.36 ± 0.05	65.5 ± 5.3	0.08 ± 0.014	0.11 ± 0.013
	70% WPH	39.8 ± 3.2	1.55 ± 0.50	5.78 ± 0.84	7.61 ± 0.23	0.25 ± 0.01	0.33 ± 0.02	58.2 ± 1.3	0.08 ± 0.012	0.11 ± 0.003
	WPH	54.3 ± 1.1	1.80 ± 0.03	4.48 ± 0.14	6.73 ± 0.14	0.19 ± 0.02	0.29 ± 0.03	42.7 ± 4.1	0.06 ± 0.002	0.10 ± 0.002
	Overlimed WPH	48.3 ± 5.8	1.04 ± 0.50	4.41 ± 0.51	5.83 ± 0.24	0.21 ± 0.04	0.28 ± 0.01	43.8 ± 9.4	0.06 ± 0.007	0.08 ± 0.003
	Activated charcoal treated WPH	49.5 ± 2.6	2.29 ± 0.28	6.27 ± 0.54	8.98 ± 0.35	0.20 ± 0.01	0.28 ± 0.01	64.1 ± 4.2	0.09 ± 0.007	0.13 ± 0.005
	Resin treated WPH	44.4 ± 1.0	1.94 ± 0.01	9.14 ± 0.82	11.35 ± 0.74	0.31 ± 0.02	0.39 ± 0.02	65.6 ± 0.4	0.13 ± 0.011	0.16 ± 0.010
	Control (glucose/xylose)	38.0 ± 1.6	2.59 ± 0.56	10.59 ± 0.87	13.67 ± 0.16	0.32 ± 0.01	0.42 ± 0.02	85.6 ± 2.0	0.15 ± 0.012	0.20 ± 0.002
Bioreactor	70% WPH	37.6	1.99	5.58	7.85	0.23	0.33	63.9	0.08	0.11
Bioreactor with gas stripping	70% WPH	41.8	3.23	9.38	12.89	0.29	0.39	78.0	0.13	0.17
	Resin and evaporation treated WPH	54.4	4.17	13.46	17.73	0.32	0.44	74.6	0.19	0.25

sugarcane bagasse hydrolysate after overliming treatment at 60 °C (Martinez et al., 2001). Overall, overliming was not good while resin adsorption was the best for treating WPH. Nevertheless, even the best ABE production (11.35 g/L with a yield of 0.39 g/g and productivity of 0.16 g/L h) from WPH detoxified with resin adsorption was still inferior to that of the control fermentation (13.67 g/L ABE with yield of 0.42 g/g and productivity of 0.20 g/L h) (see Table 2). Also, the sugar conversion was only 65.6%

(vs. 85.6% for the control) using the detoxified WPH. The relatively low sugar conversion suggested that cells were more sensitive to butanol toxicity in the presence of residual inhibitors in the detoxified WPH. More comprehensive study on the WPH inhibitors and their compounded effects on ABE fermentation would be needed in order to optimize the detoxification treatment. Furthermore, both detoxification and dilution of WPH may be necessary for achieving good ABE production with a high sugar conversion.

3.2. ABE production from WPH in the bioreactor

WPH, after dilution or detoxificatioin, was studied for ABE fermentation in a 5-L bioreactor. Based on the results from the serum bottle study, 70% diluted WPH was chosen for the bioreactor demonstration for its higher initial sugar concentration and good solvent production. Fig. 2 shows the typical fermentation kinetics with the untreated WPH diluted to 70%. About 63.9% of total sugars in the WPH were consumed, producing a total of 7.85 g/L of ABE, of which 5.58 g/L was butanol, in ~70 h. The overall ABE yield was 0.33 g/g and butanol yield was 0.23 g/g. It should be noted that *C. beijerinckii* CC101 utilized both glucose and xylose simultaneously with no obvious preference between them, but did not seem to use arabinose, which remained almost unchanged at ~1 g/L throughout the fermentation. Both sugar utilization and ABE production slowed down significantly after 55 h due to inhibition from both butanol and inhibitors present in WPH. In general, the fermentation results in the bioreactor were consistent with those obtained in serum bottles and confirmed that butanol toxicity was more severe in the presence of WPH inhibitors. The cytotoxicity of butanol, which is known to severely compromise the integrity of cell membrane, and its inhibition effect on ABE fermentation could be compounded by the WPH inhibitors, which might also compromise membrane integrity and negatively affect some membrane-bound enzymes (Ezeji et al., 2010).

3.3. ABE fermentation with gas stripping for butanol separation

To improve sugar conversion and butanol production, gas stripping as an online product recovery technique was employed to relieve butanol inhibition in the ABE fermentation. Gas stripping removes only the volatile solvents, not nutrients, and would not harm cells in the fermentation (Ezeji et al., 2004; Xue et al., 2012). As shown in Fig. 3, a total of 12.89 g/L of ABE was produced, of which 9.38 g/L was butanol, from 70% WPH in the fermentation with gas stripping starting at ~24 h. The ABE yield was 0.39 g/g and butanol yield was 0.29 g/g. Compared to the fermentation

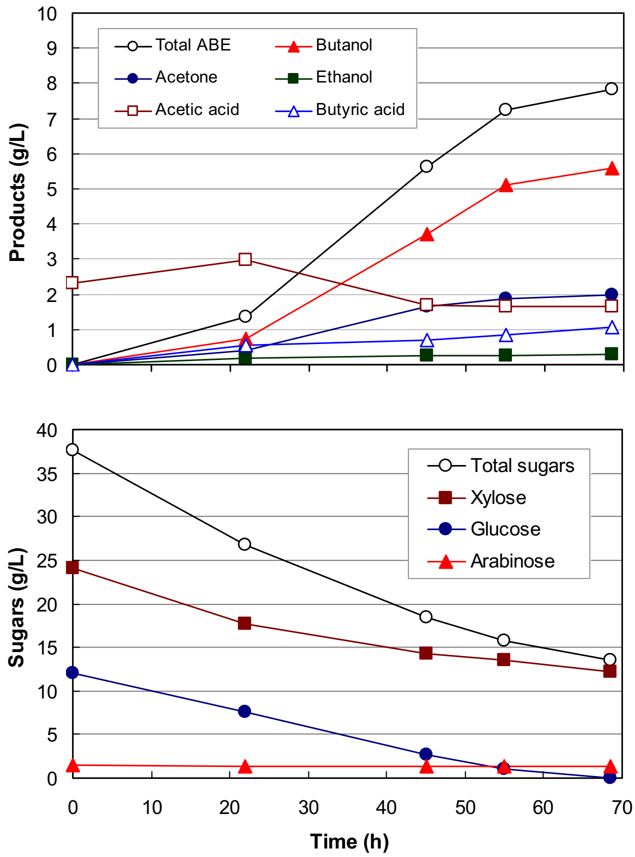

Fig. 2. Kinetics of ABE fermentation of 70% WPH by *C. beijerinckii* CC101 in a stirred-tank bioreactor at pH 5.0, 37 °C.

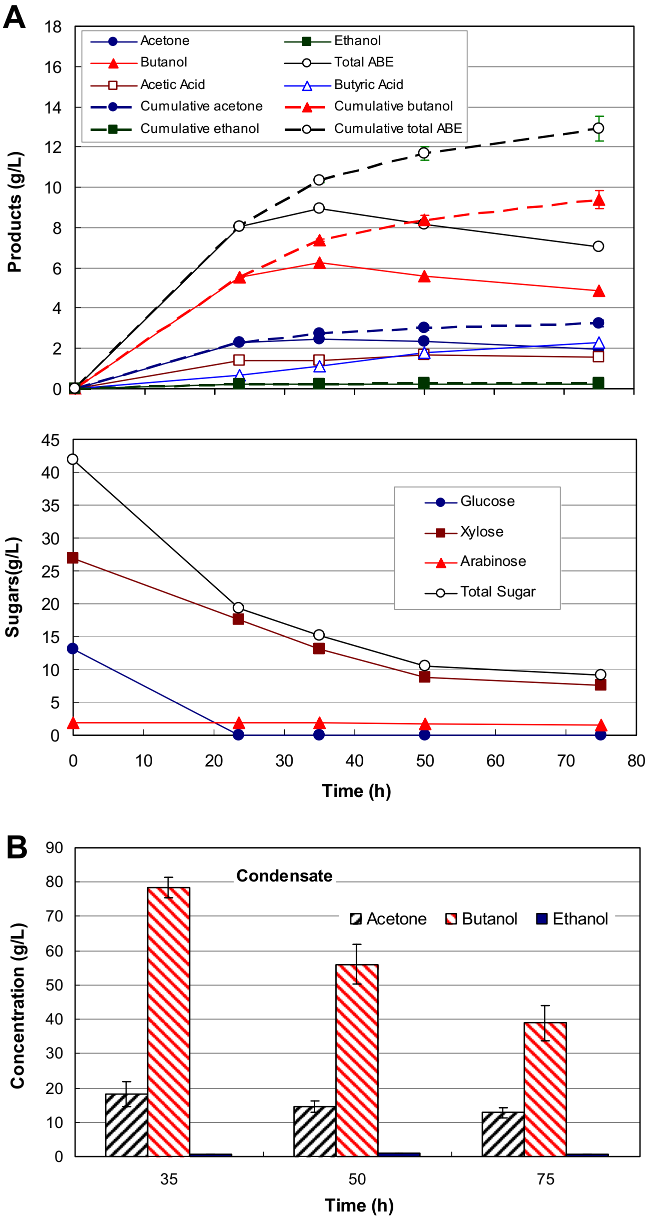

Fig. 3. Kinetics of ABE fermentation of 70% WPH by *C. beijerinckii* CC101 in a stirred-tank bioreactor with gas stripping. (A) Concentration of sugars, solvents and acids in the fermentation broth (solid lines) and cumulated production of solvents (dashed lines); (B) Concentrations of solvents in the condensates from gas stripping collected at different time points.

shown in Fig. 2, gas stripping increased the butanol production by 68.1%, with sugar utilization also increased significantly (78.0% vs. 63.9%). Similarly, ABE fermentation with online gas stripping was also studied using resin-detoxified WPH and the results are shown in Fig. 4. A total of 17.73 g/L ABE was produced, of which 13.46 g/L was butanol. The ABE yield was 0.44 g/g and butanol yield was 0.32 g/g, both were similar to those from the control. This was the best fermentation result obtained from WPH and represented a 12.1% increase in sugar utilization, which was 74.6%, and a 47.2% increase in butanol production compared to 9.14 g/L butanol produced in serum bottles without gas stripping using the same medium. Although gas stripping was effective in keeping the butanol concentration at ~6 g/L, the fermentation stopped producing any solvents after 70 h before all sugars were consumed, indicating culture degeneration, which was probably caused by WPH inhibitors and cumulated metabolites. Although butanol stress on the cells was alleviated through its removal, continued exposure to inhibitors and toxic metabolites (mainly butanol and acetone) even at reduced concentration levels might have induced cell autolysis and sporulation contributing to the early fermentation cessation (Ezeji et al., 2004).

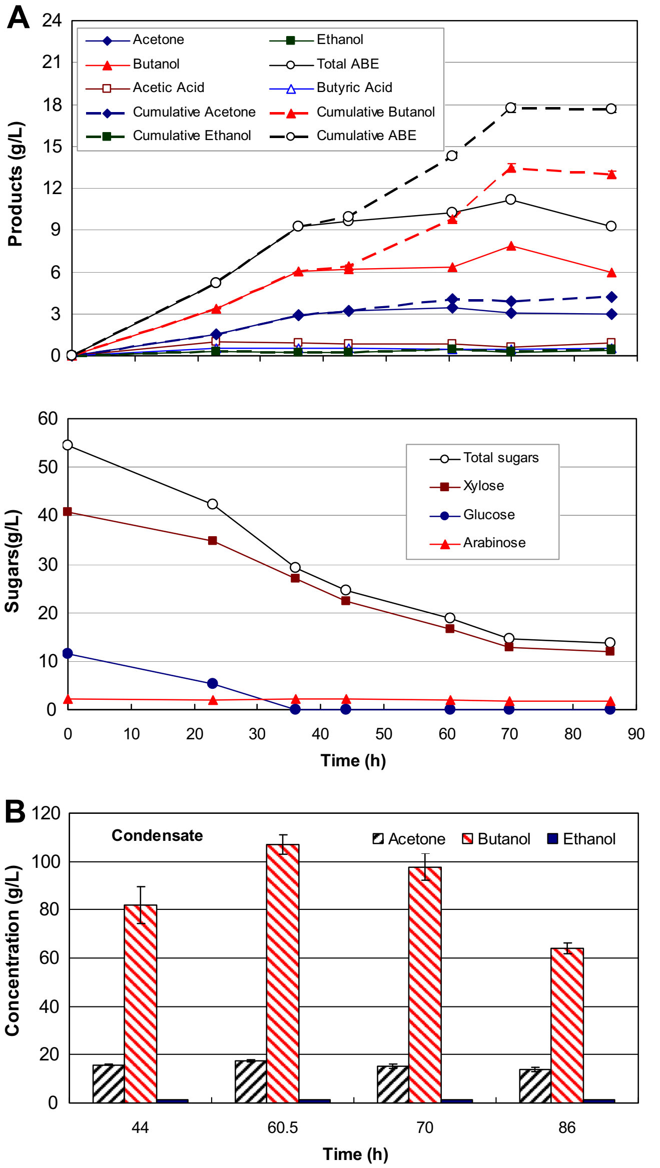

Fig. 4. ABE fermentation of resin detoxified WPH by *C. beijerinckii* CC101 in a stirred-tank bioreactor with gas stripping. (A) Concentrations of sugars, solvents and acids in the fermentation broth (solid lines) and cumulated production of solvents (dashed lines); (B) Concentrations of solvents in the condensates from gas stripping collected at different time points.

Table 3
ABE removal by gas stripping in the integrated fermentation process (2-L medium volume).

Substrate	Solvent type	Removed by stripping (g)	Produced in total (g)	Ratio of removal (removed/produced)
70% WPH	Acetone	2.54	6.46	0.39
	Ethanol	0.13	0.57	0.23
	Butanol	9.06	18.76	0.48
Resin treated WPH	Acetone	2.45	8.34	0.29
	Ethanol	0.21	0.95	0.22
	Butanol	14.15	26.92	0.53

3.4. Effects of gas stripping

In this study, gas stripping, which was carried out by recirculating the fermentation-produced gases (H_2 and CO_2) through the fermentation broth, kept the butanol concentration at ~6 g/L (Figs. 3A and 4A) while butanol was continuously recovered in the condensate at a high concentration of ~80 g/L (Figs. 3C and 4C). Acetone and ethanol were also recovered by gas stripping, but they were present at much lower concentrations in the condensate. In general, the butanol concentration in the condensate was ~80 to 110 g/L, which was 4- to 5-fold higher than that of acetone (~15 to 20 g/L), indicating a higher selectivity for butanol than acetone in the gas stripping process. Meanwhile, only a low level of ethanol (~1 g/L) was present and no acids were detected in the condensate. As shown in Table 3, a total of 18.76 g butanol was produced from 70% WPH medium in the integrated process, of which 9.06 g or 48% of the produced butanol was removed by gas stripping. In contrast, only 39% and 23% of acetone and ethanol, respectively, and no acids produced in the fermentation were removed by gas stripping. Similarly, gas stripping removed 53% of butanol, 29% of acetone and 22% of ethanol produced in the fermentation with the resin treated WPH. By selectively removing the solvents (mainly butanol and acetone) produced in the ABE fermentation, gas stripping might have also stimulated solvents production as indicated by the higher ABE and butanol yields compared to the similar fermentations without gas stripping (see Table 2).

In the gas stripping process, the solvent removal rate and selectivity over water are directly related to the solvent concentration in the fermentation broth (Xue et al., 2012). As shown in Fig. 5, a slight increase in the butanol concentration in the fermentation broth from 5 to 6 g/L doubled the butanol concentration in the condensate from ~40 to ~80 g/L, whereas the acetone concentration in the condensate remained almost unchanged at ~18 g/L when the acetone concentration in the broth increased from 2 to 4 g/L. Clearly, gas stripping is highly selective towards butanol, and is especially efficient in concentrating butanol from the ABE fermentation broth containing 6 g/L or higher of butanol to a concentration much higher than the butanol solubility (7.7% w/w) in water.

Butanol and water form an azeotrope containing 55.5% (w/v) butanol, which separates into two phases – upper phase containing 79.9% (w/w) butanol and lower phase containing 7.7% (w/w) butanol (Vane, 2008). Because of the relatively high boiling point and low volatility of butanol to water, the separation of butanol from water in a multi-column distillation process is extremely energy-intensive and requires ~79 MJ/kg of butanol (>99.9%) produced from a 0.5% (w/v) butanol-water solution (Matsumura et al., 1988). However, if the butanol concentration in the solution is increased to ~4–5% (w/v), the energy requirement for butanol

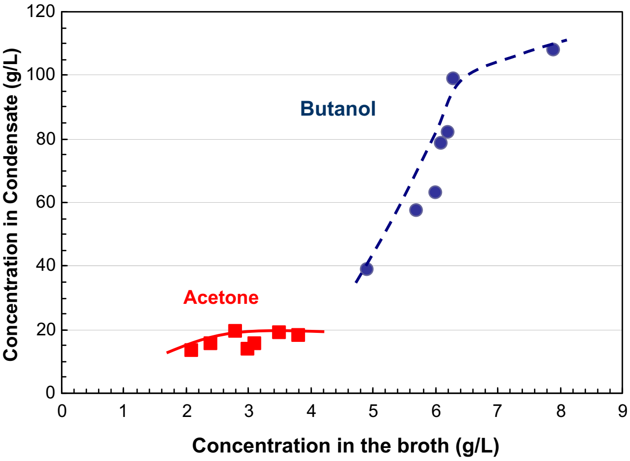

Fig. 5. Relationships between the concentrations of acetone and butanol in the fermentation broth and in the condensate from gas stripping.

recovery would be reduced to less than 10 MJ/kg, which is less than 1/3 of the energy content of butanol (36 MJ/kg butanol). When the butanol concentration is higher than 15% (w/v), the energy requirement would be reduced to ~2.6 MJ/kg of butanol (Vane, 2008). Therefore, it is advantageous to use gas stripping to concentrate butanol from the dilute ABE fermentation broth before further purification by distillation. It has been estimated that the total energy requirement for butanol recovery from the ABE broth containing ~5 g/L butanol would be 14–31 MJ/kg by gas stripping followed with distillation of the condensate containing 5–7% butanol (Oudshoorn et al., 2009). In this study, 8–11% (w/v) of butanol was obtained in the recovered condensate (Fig. 4C), resulting in a butanol-rich upper organic phase containing more than 64% (w/v) of butanol and a lower aqueous phase with ~8% (w/v) of butanol after spontaneous phase separation. Under this condition, the total energy requirement for butanol recovery would be less than 14 MJ/kg butanol (Xue et al., 2012).

In order to produce butanol-rich condensate that can phase-separate, the butanol concentration in the fermentation broth must be maintained at >6 g/L, which could be problematic to the sporulating strain, such as the one used in this study, for an extended fermentation period (see Figs. 3 and 4). However, a non-sporulating strain with higher butanol tolerance can solve this problem and has been successfully used for continuous butanol production over an extended period in fed-batch fermentation, where the butanol concentration was maintained at ~8 g/L in the fermentation broth while gas stripping produced a condensate containing butanol at >150 g/L (Lu et al., 2012; Xue et al., 2012).

3.5. Comparison to other studies

Several lignocellulosic feedstocks have been investigated for butanol production via ABE fermentation in recent years. However, only a few studies on fermenting wood hydrolysate into butanol have been reported (see Table 4). Among the lignocellulosic biomass studied to date, wheat straw hydrolysate is the least inhibitory feedstock for ABE fermentation, resulting in 25.0 g/L of ABE without detoxification (Qureshi et al., 2007, 2010a). Other hydrolysates all require detoxification and/or dilution prior to fermentation, which would otherwise result in poor cell growth and low solvent production. Some hydrolysates, for example, corn fiber hydrolysate, did not yield high solvent production even after detoxification (Qureshi et al., 2008). Evidently, some biomass feedstocks are more challenging to handle than others. In particular, fermenting the untreated wood hydrolysate has not been successful, and detoxification is necessary (Maddox and Murray, 1983). This is because the wood pulp has a high lignin content, which could result in a high level of phenolic compounds formed during the pretreatment (Kumar et al., 2009; Saha, 2003). Phenolic compounds are more toxic than sugar degradation products such as furfural and HMF, and can significantly reduce cell growth and sugar assimilation during fermentation (Palmqvist and Hahn-Hägerdal, 2000). Furfural and HMF at concentrations below 1.0 g/L were reported to be non-toxic; instead, they could stimulate ABE fermentation (Ezeji et al., 2007). Formic acid was also reported as non-inhibitory to *C. beijerinckii* at concentrations below 1.0 g/L (Cho et al., 2012). Levulinic acid was reported to increase the membrane permeability (Heipieper et al., 1994) and showed some inhibition on the ethanol production by *S. cerevisiae* at 0.5 g/L and severely inhibited the yeast at 2.5 g/L (Meinita et al., 2012). However, levulinic acid at ~1 g/L did not seem to affect the ABE fermentation as found in the present study.

Studies using activated charcoal detoxification showed that it was effective in removing organic acids at low pH and phenolic compounds at high temperature from lignocellulosic hydrolysates (Rodrigues et al., 2001; Ravi et al., 1998). It was reported that 48.9% color and 25.8% lignin degradation products were removed from rice straw hydrolysate after activated charcoal detoxification (Mussatto and Roberto, 2004). Overliming was reported to be effective in removing inhibitors and improving ABE production from some lignocellulosic biomass such as barley straw and bagasse hydrolysates (Martinez et al., 2001; Qureshi et al., 2010a,b),

Table 4
ABE fermentations with lignocellulosic biomass hydrolysates for butanol production.

Lignocellulosic biomass	Pretreatment & hydrolysis	Detoxification method	Butanol (g/L)	ABE (g/L)	Reference
Pinus radiata	Steaming followed by dilute acid hydrolysis	Activated carbon	1.6	NR	Maddox and Murray (1983)
		Anion and cation exchange resin	5.7	NR	
Aspenwood chips	Steam explosion followed by dilute acid hydrolysis	Ion-exchange resin + vacuum concentration	8.98 (max)	13.15	Yu et al. (1984)
Wood pulp	Alkaline extraction followed by dilute acid hydrolysis	Activated charcoal	6.27	8.98	This work
		Resin adsorption	9.14	11.35	
		Resin adsorption, with gas stripping for product removal	13.46	17.73	
Barley straw	Dilute acid and enzymatic hydrolysis	Overliming	18.01 (max)	26.64	Qureshi et al. (2010a)
Corn stover	Dilute acid and enzymatic hydrolysis	Overliming	14.5 (max)	26.27	Qureshi et al. (2010b)
Switchgrass	Dilute acid and enzymatic hydrolysis	Overliming	9.55 (max)	14.61	Qureshi et al. (2010b)
Corn fiber	Dilute acid and enzymatic hydrolysis	Overliming + nonionic resin	6.4	9.3	Qureshi et al. (2008)
Wheat straw	Dilute acid and enzymatic hydrolysis	None	NR	25.0	Qureshi et al. (2007)
Cassava bagasse	Enzymatic hydrolysis	Gas stripping, fed-batch fermentation	76.4	108.5	Lu et al. (2012)

NR: Not reported.

but it did not improve the fermentability of WPH by *C. beijerinckii* in our study. This suggests that each type of biomass is unique in its composition, and the respective hydrolysate requires a specific detoxification in order to be successfully used in the subsequent fermentation.

In this study, we found that activated charcoal was effective in removing furfural (80.0%), HMF (87.9%), levulinic acid (99.9%) and phenolic compounds (99.9%), and it increased butanol production by 40% using WPH. Nevertheless, resin adsorption was found to be the most effective method in detoxifying WPH, removing 100% of acetic acid, 99.9% of HMF and 57.9% of phenolics, resulting in the best butanol production in the ABE fermentation in this study. It was also demonstrated to be more effective than activated charcoal in removing the inhibitors in other studies using wood hydrolysate (Maddox and Murray, 1983; Yu et al., 1984). Larsson et al. (1999) conducted a comparison study among popular detoxification methods and reported that resin adsorption was the most effective one, removing 96% acetic acid, 73% furan, 70% HMF and 91% phenolic compounds from spruce hemicellulose hydrolysate. Wood hydrolysate detoxified by resin adsorption was also reported to improve the ethanol yield and productivity in yeast fermentation (Nilvebrant et al., 2001).

In this study, using resin detoxified WPH, 9.14 g/L butanol was obtained in batch fermentation and it was further improved to 13.46 g/L in the gas stripping integrated fermentation process. To our best knowledge, this study was the first one to demonstrate the feasibility of producing butanol from WPH in ABE fermentation with integrated gas stripping to recover butanol and alleviate butanol toxicity. WPH as an alternative feedstock to the current food-based substrates should help to improve the process economics of the ABE fermentation. It should be noted that the nutrients and nitrogen source present in the P2 medium used in this study can be replaced with low-cost industrial materials such as corn steep liquor that gave similar ABE fermentation performance (data not shown). However, further process optimization would be necessary before commercial application. The energy advantages of gas stripping and energy consumption in concentrating the WPH and its effects on process economics also need further study.

4. Conclusion

The fermentability of WPH can be improved by dilution and detoxification. Resin detoxification was more effective than overliming and activated charcoal detoxification in removing most of the inhibitors from the hydrolysate, resulting in improved ABE production to 11.35 g/L in batch fermentation by *C. beijerinckii* CC101. With simultaneous butanol recovery by gas stripping, total ABE production from WPH was further increased to 17.73 g/L in the integrated process. In summary, WPH after resin detoxification can be effectively utilized as a renewable feedstock in ABE fermentation integrated with gas stripping, overcoming a major challenge in using lignocellulosic feedstock for butanol production.

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